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FLÁVIA FUSCO VEIGA

AÇÃO DO CARBONATO DE LÍTIO SOBRE OS ÁCINOS DE PARÓTIDAS

Curitiba
2015

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Mestre em Odontologia, Área de Concentração em Biociências.

Orientadora: Profa. Dra. Ana Maria Trindade Grégio.
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ARTIGO EM PORTUGUÊS

Ação do carbonato de lítio sobre os ácinos de parótidas

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Resumo

Objetivo: O objetivo deste estudo é quantificar o número (N) e aferir o volume (V) de células acinares de glândulas parótidas, bem como o tamanho e massa daquela glândula em ratos submetidos cronicamente ao carbonato de lítio, mimetizando uma condição de tratamento do distúrbio bipolar.

Desenho: Vinte e cinco ratos machos Wistar foram distribuídos em dois grupos de acordo com a administração de: a) (n=11) controle com solução salina aplicação diária via intraperitoneal, por 51 dias; b) (n=14) carbonato de lítio (60 mg/kg mesma frequência e via). A glândula parótida direita de cada animal foi removida, pesada, medida e processada, e os cortes histológicos corados pela hematoxilina e eosina, a partir da qual o N e o V foram quantificados. Para análise estatística foi utilizado o teste t de Student.

Resultados: Foi verificado, que com o uso do lítio, houve um aumento estatisticamente significante no tamanho (1,00 ± 0,20 cm) e na massa glandular (150,71±73,95 mg) quando comparado com o controle (0,80±0,17 cm e 108,93±17,00 mg respectivamente) ($p<0,05$). E houve equivalência em volume e número celular.

Conclusão: Concluiu-se que o Li^+ não modifica o número e volume celulares, indicando que não ocorreu alteração morfométrica no parênquima glandular. Além disso, o maior tamanho e massa da glândula poderiam sugerir que estroma glandular encontra-se edemaciado e isto poderia acarretar em hipossalivação.

Pontos chave:

- O carbonato de lítio é um estabilizador de humor que pode causar a hipossalivação.
- A hipossalivação interfere negativamente na qualidade de vida dos pacientes.
- Foi investigado alterações morfométricas na parótida submetida ao carbonato de lítio.
- O lítio não altera o número e volume celular acinar.
- O lítio aumenta o tamanho e a massa da glândula parótida.

Palavras-chave: Lítio, glândula salivar, hipossalivação, morfometria, glândulas parótidas, parênquima glandular.

Introdução

A informação e os avanços tecnológicos propiciaram uma melhor qualidade de vida e longevidade. Por outro lado, se observa um aumento expressivo no número de indivíduos afetados por distúrbios que até então haviam sido pouco estudados. Entre esses, estão os distúrbios afetivos, que se caracterizam por alterações de humor com depressão, distúrbios bipolares e distúrbios do pensamento[1].

Para o tratamento desses distúrbios pode se destacar o carbonato de lítio, que foi o primeiro fármaco aprovado pela Food and Drug Administration (FDA) para o tratamento do transtorno de humor bipolar[2] e tem sido usado por mais de 60 anos para tratamento das fases agudas e de manutenção da doença bipolar[3,4]. Atua na depressão e mania, reduz o risco de suicídio e mortalidade a curto prazo[5]. Possui propriedades estabilizadoras do humor e neuroprotetoras, que previnem apoptose dos neurônios[6].

Apesar de o lítio ser amplamente usado e ser efetivo para o tratamento das desordens de humor, ele é responsável por algumas alterações clínicas como a polidipsia, sabor desagradável, tremor, risco de efeitos teratogênicos, entre outros. Seu índice terapêutico estreito necessita de acompanhamento de rotina das concentrações séricas de hormônio e função renal[5]. Por ser uma medicação bastante prescrita, tem sido cada vez mais comum o dentista tratar pacientes que fazem uso de medicamentos para controle de distúrbios psiquiátricos e que estão sujeitos aos seus efeitos colaterais[7].

Está bem estabelecido que uma grande variedade de fármacos psicotrópicos tem efeito pronunciado na função das glândulas salivares e que causam modificações dos mecanismos de secreção associados a ativação dos receptores muscarínicos, induzindo um efeito anticolinérgico que acarreta hipossalivação[8,4,9]. A diminuição ou ausência de saliva pode afetar o estado emocional do paciente, causando um aumento da morbidade e redução da qualidade de vida[10].

Os principais sintomas relacionados com a hipossalivação são a sensação de boca seca ou xerostomia, sede frequente, dificuldades em falar, deglutir e mastigar, dificuldades em usar dentaduras, dor e irritação na mucosa. Os sinais encontrados com maior frequência são a perda da lubrificação oral, o surgimento

de fissuras na mucosa e no dorso da língua, queilite angular, halitose, alterações na percepção do gosto, aumento do número de cáries e dentes perdidos, e aumento no tamanho das glândulas salivares [29,30].

A secreção salivar é controlada pelo sistema nervoso autônomo simpático e parassimpático, estimulados através de uma sequência coordenada de trandução de sinal e eventos de sinalização intracelular. A via do simpático controla a qualidade da saliva assim como a secreção de amilase, proteínas e íons salivares. Por outro lado a via do parassimpático controla a liberação da secreção salivar em termos de quantidade. O estímulo do parassimpático ocorre via receptor muscarínico. por meio da elevação da concentração do inositol trifosfato (IP3). As etapas são orquestradas da seguinte forma: o receptor muscarínico nas células acinares parotídeas ativa a proteína G, resultando na hidrólise do IP2 pela fosfolipase C e assim produz o IP3 intracelular que estimula a liberação do Ca^{+2} dos estoques intracelulares dos ácinos, levando a uma maior concentração de cálcio livre[12]. Esse aumento da concentração intracelular de Ca^{+2} induz a ativação: a) das bombas de Cl^{-}, para equilibrar a voltagem da célula e b) do canal de K^{+} mantendo o potencial de membrana negativo, preservando o efluxo do Cl^{-} para o lúmen da glândula salivar. Sendo assim, o Na^{+} se difunde para o meio intracelular acinar mantendo a eletroneutralidade. O Na^{+} e Cl^{-} promovem um ambiente hipertônico intracelular facilitando a entrada da água para o lúmen acinar, resultando na produção de uma saliva fluida[22].

A degradação do IP3 é, também, um passo necessário na cascata do fosfato de inositol para que se finalize a resposta ao Ca^{+2}. O trifosfato é metabolizado pelas enzimas inositol polifosfato 5-fosfatase e 1,4,5-trifosfato de inositol 3-quinase, resultando em inositol-1,4-bifosfato e inositol 1,3,4,5-tetraquisfosfato. Estes são dois metabólitos que serão desfosforilados por fosfatases, gerando o inositol que junto com a citidina monofosforilfosfatidase, é o precursor inositol lipídico necessário para continuar a sinalização. O Li interfere na quantidade de fosfatidil-inositol por inibição da atividade da monofosfatase de inositol, impedindo deste modo a reciclagem de inositol. Esta ação inibitória do Li

resulta em um acúmulo de ambos os monofosfatos de inositol e citidina monofosforilfosfatidase, e pode culminar na ausência de inositol tão importante no processo de secreção salivar tanto para a liberação quanto para a produção[12].

Considerando que há uma carência de informações que discutam os mecanismos do lítio na redução do fluxo salivar, o presente estudo propõe uma avaliação histomorfométrica de células acinares parotídeas de ratos submetidos ao carbonato de lítio em concentrações iguais aquelas utilizadas no tratamento das desordens de humor bipolar.

Material e Método

Este estudo foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA) da Pontifícia Universidade Católica do Paraná (PUCPR), sob o número de registro 631/11.

Foram utilizados 25 ratos machos tipo Wistar (*Rattus norvegicus albinus, Rodentia, mammalia)*, oriundos do Biotério Central da Pontifícia Universidade Católica do Paraná e pesando aproximadamente 300 g. Os ratos foram mantidos em gaiolas plásticas com água e comida *ad libidum*, respeitando o fotoperíodo de doze horas claro/escuro, e aclimatados ao ambiente laboratorial.

Os animais foram separados aleatoriamente em dois grupos, um grupo contendo 11 animais, grupo controle e outro grupo com 14 animais, grupo experimental. Os animais receberam o tratamento por 51 dias. No GC foi administrada a salina (intraperitoneal diária) (LBS-Laborasa Indústria Farmacêutica LTDA, São Paulo, SP, Brasil) e no GL administrou-se via peritoneal, 60mg/kg/dia de carbonato de lítio em solução salina (Bioarte Farmácia de manipulação LTDA, Piracicaba, SP, Brasil). A pureza e potencia foram certificados pelo fabricante. A aplicação foi realizada por meio de seringas plásticas descartáveis agulhadas U-100 Insulin ½ cc 0,05 m (BD ultra-fineTM BD-Becton Driver, NJ).

Análise bioquímica

Após os 51 dias e, noventa minutos após a última administração de carbonato de lítio[15], o sangue dos animais foi coletado por meio de punção intracardíaca no momento da eutanásia. Foram colhidos 6 ml de sangue em tubo Vacutainer® com gel (BD- Becton Driver, NJ), descartável sem anticoagulante.

A análise bioquímica do sangue foi realizada em Laboratório de Análises Clínicas LANAC (Curitiba, PR, Brasil), sendo o nível sérico de lítio mensurado pelo analisador automático Dimension RXL (Medcorp-Siemens, Brasília, DF, Brasil).

Com essa análise verificou-se que o nível sérico de lítio no grupo experimental foi 1,30 ± 0,55 mmol/L.

Excisão da parótida e mensuração de seu tamanho

Após a conclusão de cada tratamento, os animais foram anestesiados e mortos por meio da administração intraperitoneal de tiopental sódico (Thionembutal®, Abbott laboratórios do Brasil Ltda., São Paulo, Brasil) na dose de 100 mg/kg.

A glândula parótida direita foi removida e mensurada (no sentido longitudinal) com um paquímetro digital (Mitutoyo 500 Mical®,Mitutoyo Co. Tokio, Japão) e pesada em balança de precisão Belmark® U210A (Bel Engenharia, Piracicaba, Brasil). Dessa forma, definiu-se o tamanho (T) e a massa (M) de cada glândula. O espécime foi imerso em formol a 10% por 48 horas. Cada peça foi seccionada transversalmente seguindo o sentido epitélio-conjuntivo.

Análise histomorfométrica

Os espécimes foram processados e embebidos em parafina seguindo a rotina do Laboratório de Patologia Experimental de PUCPR, e os cortes histológicos com 4 micrômetros de espessura foram corados por hematoxilina e eosina.

Para a análise histomorfométrica foram capturadas 80 imagens de cada lâmina com as objetivas de 40X e 100X (40 em cada), por meio de uma microcâmera Dinolite® AM 423X (AmMo Eletronics Corporation, New Taipei city 241, Taiwan) acoplada ao microscópio Olympus® BX 50 (Olympus Corporation, Ishikawa, Japão) e a um microcomputador (Acer, Aspire One Series, modelo ZH7). As imagens foram avaliadas em um software analisador (Image-Pro®Plus, Cybernetics, Maryland). Utilizando-se a metodologia de Onofre et al (1987), obteve-se o volume (V) e o numero celular (N), conforme descrito abaixo:

Quantificação do volume celular (V)

O volume da glândula salivar processada (Vp) foi calculado através da fórmula: Vp=m/d.rf, aonde m é a massa fresca, d a densidade e rf as alterações causadas pelo processamento morfológico. Para estes cálculos usou-se d =

1.089g/cm^2, um valor obtido pelo método de Scherle (1970), e rf = 0.7, obtido pelo método de Taga e Sesso (1978).

Em imagens capturadas em 40X aplicou-se uma grade com dez linhas horizontais e dez linhas verticais, determinando 100 pontos (Pt) simetricamente distribuídos sobre a área quadrangular. Desses foram contados quantos pontos coincidiam com o ácino (Pi). A densidade do volume acinar (Vvi) foi calculada: Vvi=Pi/Pt. O volume total acinar foi determinado através da fórmula : Vti=Vvi.Vp.

Nas imagens capturadas com a objetiva de 100X, obteve-se a média do diâmetro ortogonal de 50 núcleos. Calculou-se então, o raio de cada núcleo com a fórmula r²=d1.d2. O volume dos núcleos das células acinares foi obtido usando a fórmula Vni=4/3.¶. r^3 .

Novamente, nas imagens capturadas em 40X, o número de pontos que coincidiam com os núcleos (Pn) e com o citoplasma (Pcity) das células acinares glandulares foi contabilizado. A densidade do volume nuclear corrigida (Pncorr) foi determinda pela expressão Pncorr = (Pn / Pn + Pcity)Ko, aonde ko é o fator de correção calculado pela fórmula Ko= 1+3t/ 2d, onde d é o diâmetro de cada núcleo e t a espessura de corte da lâmina, que foi de 4 µm.

A densidade do volume do citoplasma foi corrigida através da fórmula Pcyticorr = 1 – Pncorr.

A razão citoplasma dividido pelo núcleos das células acinares (Rc/n), foi definida através de: Rc/n = Pcyticorr/Pncorr.

Para finalizar, o volume citoplasmático (Vcyti) foi calculado através da equação Vcyti= Vni.Rc/n e o volume celular (V) pela fórmula V= Vni+ Vcyti.

Quantificação do número de células acinares (N)

As imagens em 40X, com a grade previamente descrita, foram contados os números de núcleos e o número de núcleos presentes nos cruzamentos (c) entre as margens dos perfis das imagens nucleares e as linhas paralelas da grade. Também foi mensurada a área total em micrômetros quadrados (A), a distância entre as linhas da grade (d), a espessura da secção (t), com as variáveis supracitadas foi obtido o número total de núcleos de células acinares (N) de acordo com a equação:

$$N = \frac{2n \times V_p}{A \times \left(\frac{c}{n \times d} + 2 \times t \right)}$$

Análise estatística

Na análise estatística foi utilizado o programa SPSS 20.0 (SPSS Inc, Chicago, IL). O teste de normalidade de Kolmogorov-Smirnov revelou que as variáveis apresentaram distribuição normal, optando-se então pelo teste t de Student. O teste de Levene verificou que as variáveis número de células e tamanho da glândula apresentaram variância homogênea, enquanto as demais foram heterogêneas. O nível de significância adotado em todos os testes foi de 5%. Foi avaliado o poder de reprodutibilidade das variáveis e verificou-se que o erro de Dahlberg variou de 0,62 a 6,3%, indicando que o único avaliador reproduziu as medidas de forma confiável [21,22]. O teste t de Student revelou não haver erro sistemático na contagem ($p > 0,05$).

Resultados

Maior tamanho e massa da glândula foram observadas nos animais tratados com lítio. Para as demais variáveis não foram observadas diferenças significativas (Tabela 1).

Tabela 1 – Tamanho e massa da glândula, volume celular e número de núcleos nos grupos lítio e controle

Grupos/ variáveis	Lítio (média ± DP)	Controle (média ± DP)
Tamanho da glândula (cm)	1,00 ± 0,20 A	0,80±0,17B
Massa da glândula (mg)	150,71±73,95 A	108,93±17,00B
Volume celular (μm³)	1458,88±287,93 A	1711,87±508,70A
Número de células (x10^6)	46,75±25,97 A	35,58±15,04A

Teste t de Student: Letras diferentes em linha indicam diferenças estatisticamente significativas

DP = desvio padrão.

Discussão

Este é o primeiro estudo que disserta sobre o efeito do carbonato de lítio sobre a histomorfometria de glândulas parótidas de ratos. Foi verificado que nos animais tratados com lítio ocorreu: a) maior tamanho da glândula; b) maior massa glandular; c) equivalência do volume celular; d) correspondência do número de células quando comparados com o controle.

Vários são os autores que dissertam sobre a ação do lítio na redução da secreção salivar causando uma hipossalivação devido a falta do estímulo nervoso para a síntese da secreção salivar, logo se não há saliva, não tem como liberá-la[9, 12, 23].

Estudos prévios com antidepressivos e benzodiazepínicos demonstraram uma hipossalivação devido à ação anticolinérgica dos mesmos sobre os receptores alfa, beta e gaba presentes nas mesmas. Esses psicofármacos são capazes de promover na glândula parótida uma hipertrofia acinar (representada pelo maior volume celular) observada nos trabalhos de Grégio[8], Mattioli[11], Zaclikevis[25] e Silva[28]. Apesar de o lítio ser um fármaco de ação central e um psicofármaco o mecanismo de ação sobre a morfologia dos ácinos parotídeos foi diferente, já que não observou-se alteração do volume acinar. Isso indica que o lítio apresenta uma farmacodinâmica diferenciada dos outros psicofármacos, muito provavelmente sobre alguns mensageiros modificando a transdução de sinais.

Markitziu[9], com base em sialografia e cintilografia, sugeriu que a redução da taxa do fluxo salivar provocada pelo lítio poderia ser resultante de danos morfológicos e funcionais nas glândulas salivares. O presente trabalho demonstra que não houve alteração morfológica acinar, pois houve preservação do número

e volume celulares, o que indica que o dano ocorrido foi apenas funcional.

De acordo com nossos resultados a alteração funcional, sugerida pela alteração no tamanho e na massa podem ser fatores auxiliadores num processo de modificação na transdução de sinais mostrada no trabalho de Tritsaris[12], o que influenciaria em uma alteração na sequência da secreção salivar, mediada pela concentração do inositol trifosfato (IP3). O Li interfere na quantidade de fosfatidil-inositol por inibição da atividade do monofosfatase de inositol, impedindo deste modo a reciclagem de inositol. Esta ação inibitória do Li resulta em um acúmulo de ambos os monofosfatos de inositol e citidina monofosforilfosfatidase, e pode culminar na ausência de inositol tão importante no processo de secreção salivar tanto para a liberação quanto para a produção [12].

O aumento do tamanho e da massa glandular sugere que houve possivelmente a retenção de líquido (edema) no estroma glandular, já que não ocorreu alteração no parênquima, representado pela equivalência no número e volume celulares entre os grupos. O edema causado pelo uso do carbonato de lítio foi verificado em várias regiões como: pretibial, mão, abdômen, cintura pélvica, tornozelo, pálpebras[24], pulmão[25]. Esse edema pode ter sido provocado por uma alteração na redistribuição do Na^+ nos compartimentos intracelulares para o extracelular[24]. Esse edema extracelular pode ser facilitante na redução da função glandular quanto as trocas eletrolíticas, distribuição de nutrientes e oxigênio.

Concluiu-se que o Li^+ não modifica o número e volume celulares, indicando que não ocorreu alteração morfométrica no parênquima glandular. Porém a alteração em um tamanho e massa glandulares maiores, poderiam sugerir que o estroma glandular encontra-se edemaciado e isto poderia acarretar em hipossalivação.

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ARTIGO EM INGLÊS

ACTION OF LITHIUM CARBONATE ON PAROTID ACINI

Abstract

Objective: The aim of this study is to quantify the number (N) and measure the volume (V) of acinar cells of parotid glands, as well as the size and mass of that gland in rats submitted to chronic lithium carbonate, mimicking a treatment condition bipolar disorder.
Design: Twenty-five male Wistar rats were divided into two groups according to the administration of: a) (n = 11) control applied daily with saline intraperitoneally for 51 days; b) (n = 14) lithium carbonate (60 mg / kg and via the same frequency). The parotid gland of each animal was removed, weighed, measured and processed, and the histological sections stained with hematoxylin and eosin, from which the N and V were quantified. Statistical analysis was performed using Student's t test.
Results: It was found that with the use of lithium, there was a statistically significant increase in size (1.00 ± 0.20 cm) and the gland mass (150.71 ± 73.95 mg) when compared to the control (0 80 cm ± 0.17 and 108.93 ± 17.00 mg, respectively) ($p < 0.05$). And there was equivalence in volume and cell number.
Conclusion: We conclude that the Li^+ does not modify the cell number and volume, indicating that no change in morphometric glandular parenchyma. Moreover, the greater size and mass of the gland glandular might suggest that stroma is swollen and this could result in hyposalivation.

Highlights:

• Lithium carbonate is a mood stabilizer which can cause hyposalivation.
• The hyposalivation impairs the quality of life of patients.
• We investigated morphological changes in the parotid subjected to lithium carbonate.
• Lithium does not change the number and acinar cell volume.
• Lithium increases the size and weight of the parotid gland.

Key-words:

Lithium, salivary gland, hyposalivation, morphometry, parotid glands, glandular parenchyma.

Introduction

The information and technological advances have provided better quality of life and longevity. On the other hand, there is a significant increase in the number of individuals affected by disorders that have hitherto been little studied. Among these are the affective disorders, which are characterized by mood changes with depression, bipolar disorders and thought disorders[1].

For the treatment of these disorders, it can be mentioned the lithium carbonate, which has been the first drug approved by the Food and Drug Administration (FDA) for the treatment of bipolar mood disorders[2] and has been used for more than 60 years for the treatment of the acute phases and maintenance of bipolar disorders[3,4]. It acts both in depression and mania, as well as reduces the risk of suicide and short-term mortality[5]. It presents humor stabilizing and neuroprotective properties which prevent apoptosis of neurons[6].

Although lithium is widely used and is effective for the treatment of mood disorders, it also triggers some clinical disadvantages such as polydipsia, unpleasant taste, tremor, risk of teratogenic effects, among others. Its narrow therapeutic index requires routine monitoring of serum hormone concentrations and renal function[5]. For being a fairly prescribed medication, it has been increasingly common for dentists to treat patients who use medicines to control psychiatric disorders and that are subjected to its collateral effects[7].

It is well established that a variety of psychotropic drugs have pronounced effects on the function of the salivary glands and that cause changes in the secretion mechanisms associated with the activation of muscarinic receptors, inducing an anticholinergic effect which causes xerostomia[8,4,9]. The reduction or absence of saliva can affect the emotional state of the patient, causing increased morbidity and reduced quality of life[10].

The salivary secretion is controlled by the sympathetic and parasympathetic autonomic nervous system. The sympathetic pathway controls the saliva's quality, as well as the secretion of amylase, proteins and salivary ions. On the other hand, the parasympathetic pathway controls the salivary secretion release in terms of quantity. The parasympathetic stimulation occurs via muscarinic receptor, by elevating the IP3 concentration. The steps are

orchestrated as follows: the muscarinic receptor in parotid acinar cells activates the G protein, resulting in IP2 hydrolysis by phospholipase C, and so produces the intracellular IP3 that stimulates the Ca^{2+} release from intracellular stores of acini, leading to a higher concentration of free calcium[12]. This increase in the Ca^{2+} intracellular concentration induces the activation of: a) Cl^- pumps, to balance the voltage of the cell and b) K^+ channel, maintaining the negative membrane potential, preserving the Cl^- efflux into the lumen of the salivary gland. Therefore, the Na^+ diffuses through the intracellular acinar maintaining electroneutrality. Na^+ and Cl^- promote a hypertonic intracellular environment, facilitating the entry of water into the acinar lumen, resulting in the production of fluid saliva[22].

The IP3 degradation is also a necessary step in the inositol phosphate cascade to finalize the response to the Ca^{2+}. The triphosphate is metabolized by enzymes inositol polyphosphate 5-phosphatase and 1,4,5-triphosphate of inositol 3-kinase, resulting in inositol-1,4-bisphosphate and inositol 1,3,4,5-tetrakisfosfato. These are two metabolites that are dephosphorylated by phosphatases, generating the inositol, which along with the cytidine monofosforilfosfatidase, is the inositol lipid precursor necessary to continue signaling. The Li interferes with the amount of phosphatidylinositol by inhibiting the activity of inositol monophosphatase, thereby preventing the recycling of inositol. This inhibitory action of Li results in an accumulation of both inositol monophosphates and cytidine monofosforilfosfatidase, and can culminate in the absence of inositol, so important in the salivary secretion process, both for production and for release[12].

Whereas there is a lack of information to discuss the mechanisms of lithium in reducing the salivary flow, this study proposes a histomorphometric evaluation of the parotid acinar cells of rats subjected to lithium carbonate.

Material and Method

This study was approved by the Ethics Committee on the Use of Animals (CEUA - *Comitê de Ética no Uso de Animais*) of the Pontifical Catholic University

of Paraná (PUCPR - *Pontifícia Universidade Católica do Paraná*), under the 631/11 registration number.

We used 25 male rats, Wistar type (*Rattus norvegicus albinus, Rodentia, mammalia),* from the Central Animal Facility of the Pontifical Catholic University of Paraná, and weighing approximately 300g. The rats were kept in plastic cages with water and food *ad libitum*, respecting the 12 hours light/dark photoperiod, and acclimated to the laboratory environment.

The animals were randomly separated into two groups, one group containing 11 animals - Control Group - and another group containing 14 animals - Experimental Group. The animals received the treatment for 51 days. In the CG, saline was administered (daily intraperitoneal) (LBS-Laborasa Indústria Farmacêutica LTDA, São Paulo, SP, Brazil) and in the LG it was administered via peritoneal, 60 mg/kg/day of lithium carbonate in saline solution (Bioarte Farmácia de Manipulação LTDA, Piracicaba, SP, Brazil). Purity and potency have been certified by the manufacturer. The application was performed through disposable plastic syringes needles U-100 Insulin ½ cc 0,05 m (BD ultra-fineTM BD- Becton Driver, NJ).

Biochemical Analysis

After 51 days and 90 minutes of the last administration of lithium carbonate[15], the animals' blood was collected through intracardiac puncture at the time of euthanasia. 6 ml of blood were collected into Vacutainer® tube with gel (BD-Becton Driver, NJ), disposable without anticoagulant.

The biochemical blood analysis was conducted in LANAC Clinical Analysis Laboratory (Curitiba, PR, Brazil), and the serum levels of lithium were measured by the Dimension RXL automatic analyzer (Medcorp-Siemens, Brasília, DF, Brazil).

With this analysis, it was found that the serum level of lithium in lithium group was 1.30 ± 0.55 mmol/L.

Excision of the parotid and measurement of its size

Upon completion of each treatment, the animals were anesthetized and killed by intraperitoneal administration of sodium thiopental (Thionembutal®, Abbott Laboratórios do Brasil Ltda., São Paulo, Brazil) at a dose of 100 mg/kg.

The right parotid gland was removed and measured (lengthwise) with a digital caliper (Mitutoyo 500 Mical®, Mitutoyo Co. Tokio, Japan) and weighed on a

precision scale Belmark® U210A (Bel Engenharia, Piracicaba, Brazil). Thus, it was defined the size (T) and the mass (M) of each gland. The specimen was immersed in 10% formalin for 48 hours. Each piece was sectioned transversely following the epithelium-connective sense.

Histomorphometric analysis

The specimens were processed and embedded in paraffin following the routine of the PUCPR Experimental Pathology Laboratory, and the 4 micrometers thick histological sections were stained by hematoxylin and eosin.

For the histomorphometric analysis, 80 images of each blade were captured with 40X and 100X lenses (40 in each), through a Dinolite® AM 423X microcam (AmMo Electronics Corporation, New Taipei city 241, Taiwan) attached to the Olympus® BX 50 microscope (Olympus Corporation, Ishikawa, Japan) and a computer (Acer, Aspire One Series, model ZH7). The images were evaluated in an analyzer software (Image-Pro®Plus, Cybernetics, Maryland). Using the Onofre et al (1987) methodology, we obtained the cellular volume (V) and number (N), as described below:

Cellular volume quantification (V)

The processed salivary gland volume (Vp) was calculated using the formula: Vp=m/d.rf, where m is the fresh mass, d is the density and rf are the changes caused by morphological processing. For these calculations we used d = 1,089g/cm^2, a value obtained by the Scherle method (1970), and rf = 0,7, obtained by the Taga and Sesso method (1978).

In images captured in 40X was applied a grid with ten vertical lines and ten horizontal lines, determining 100 points (Pt) symmetrically distributed over the quadrangular area. From these were counted how many points matched the acini (Pi). The acinar volume density (Vvi) was calculated: Vvi=Pi/Pt. The total acinar volume was determined using the formula: Vti=Vvi.Vp.

In the images captured with the 100X lens, we obtained the average of the orthogonal diameter of 50 nuclei. It has been estimated then the radius of each nucleus using the formula r²=d1.d2. The volume of the acinar cells' nuclei was obtained using the formula Vni=4/3.¶. r^3 .

Again, in the images captured at 40X, the number of points coinciding with the nuclei (Pn) and the cytoplasm (Pcity) of glandular acinar cells was recorded. The corrected density of the nuclear volume (Pncorr) was determined by the

expression Pncorr = (Pn / Pn + Pcity)Ko, where ko is the correction factor calculated by the formula Ko= 1+3t/ 2d, where d is the diameter of each nucleus and t is the blade section thickness, which was 4 μm.

The cytoplasm volume density was corrected through the formula Pcyticorr = 1 – Pncorr.

The reason cytoplasm divided by the acinar cells' nuclei (Rc/n) was defined by: Rc/n = Pcyticorr/Pncorr.

Finally, the cytoplasmic volume (Vcyti) was calculated by the equation Vcyti= Vni.Rc/n and the cell volume (V) by the formula V= Vni+ Vcyti.

Acinar cell number quantification (N)

In the 40X images, with the grid previously described, were counted the numbers of nuclei and the number of nuclei present at intersections (c) between the profiles' edges of the nuclear images and the parallel lines of the grid. It was also measured the total area in square micrometers (A), the distance between the grid lines (d), the thickness of the section (t), and with the aforementioned variables, the total number of acinar cells' nuclei (N) was obtained, according to the equation:

$$N = \frac{2n \times V_p}{A \times \left(\frac{c}{n \times d} + 2 \times t \right)}$$

Statistical analysis

The statistical analysis was performed using the program SPSS 20.0 (SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov normality test revealed the variables were normally distributed, choosing then for the Student's t test. The Levene test found that the variables cell number and gland size presented homogeneous variance, while the others were heterogeneous. The significance level adopted in all tests was 5%. We evaluated the variables reproducibility power and found that the Dahlberg error ranged from 0.62 to 6.3%, indicating that the single evaluator reproduced reliably measures [21,22]. The Student's t test revealed no systematic error in the count ($p > 0.05$).

Results

Greater size and mass of the gland were observed in animals treated with lithium. For the other variables, no significant differences were observed (Table 1).

Table 1 – Size and mass of the gland, cellular volume and number of nuclei in the groups lithium and control

Groups/ variables	Lithium (average ± DP)	Control (average ± DP)
Gland size (cm)	1.00 ± 0.20 A	0.80±0.17B
Gland mass (mg)	150.71±73.95 A	108.93±17,00B
Cellular volume (μm³)	1458.88±287.93 A	1711.87±508.70A
Cell number (x10^6)	46.75±25.97 A	35.58±15.04A

Student's t test: Different letters in line indicate statistically significant differences

DP = standard deviation.

Discussion

This is the first study that discourses about the effect of lithium carbonate on the histomorphometry of rats' parotid glands. It was verified that in the animals treated with lithium, occurred: a) larger size of the gland; b) bigger glandular mass; c) equivalence of cellular volume; d) matching the number of cells when compared to the control.

There are several authors[9, 23,12] who discourse on the action of lithium in reducing salivary secretion causing a hyposalivation due to the lack of nerve stimulation for the synthesis of salivary secretion. So, if there is no saliva, there is no release of it.

Previous studies with antidepressants and benzodiazepines demonstrated a hyposalivation due to anticholinergic effects thereof on the alpha, beta and gaba receptors, present in them. These psychotropic drugs are able to promote acinar hypertrophy on parotid gland (represented by the largest cellular volume) observed in studies of Grégio[8], Mattioli[11], Zaclikevis[25] and Silva[28]. Although lithium

is a centrally acting drug and a psychotropic drug, the mechanism of action on the morphology of the parotid acini was different, since there was no change in the acinar volume. This indicates that lithium presents a different pharmacodynamics of other psychotropic drugs, most likely on some messengers, by modifying the signal transduction.

Markitziu et al. (1988), based on sialography and scintigraphy, suggested that the reduction in the salivary flow rate caused by lithium could be resulted from morphological and functional damages occurring in the salivary glands' acini. This study demonstrates that there was no acinar morphological change, because there was the preservation of the cell number and volume, indicating that there was just a functional damage.

According to our findings, the functional change, suggested by the size and mass changes, can be helper factors in the signal transduction modification process shown in Tritsaris work, that would influence in a change in the sequence of salivary secretion, which is mediated by the IP3 concentration. The Li interferes with the amount of phosphatidylinositol by inhibiting the activity of inositol monophosphatase, thereby preventing the recycling of inositol. This inhibitory action of Li results in an accumulation of both inositol monophosphates and cytidine monofosforilfosfatidase, and can culminate in the absence of inositol, so important in the salivary secretion process, both for production and for release[12].

The increased glandular size and mass suggests that there was possibly liquid retention (edema) in the glandular stroma, since there was no change in the parenchyma, represented by the equivalence in cell number and volume between groups. The edema caused by the use of lithium carbonate has been verified in several regions, such as: pretibial, hand, abdomen, pelvic girdle, ankle, eyelids[24], lung[25]. This edema may have been caused by a change in the redistribution of Na^+ in the intracellular compartments for the extracellular[24]. This extracellular edema may be facilitating the reduction of glandular function in relation to electrolyte changes, distribution of nutrients and oxygen.

It was concluded that Li^+ does not modify the cell number and volume,

indicating that there was no morphometric change in the glandular parenchyma. In addition, the greater size and mass of the gland might suggest that the glandular stroma is swollen and this could result in hyposalivation.

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ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA EM USO DE ANIMAIS

Pontifícia Universidade Católica do Paraná
Núcleo de Bioética
Comitê de Ética no Uso de Animais
PUCPR

Curitiba,11 de agosto de 2011.

PARECER DE PROTOCOLO DE PESQUISA

REGISTRO DO PROJETO: 631 – 1ª versão
TÍTULO DO PROJETO: Efeito do carbonato de lítio no movimento dentário induzido em ratos
PESQUISADOR RESPONSÁVEL: Viviane da Silva Kagy

EQUIPE DE PESQUISA:
Viviane da Silva Kagy, Aline Cristina Batista Rudrigues, Elisa de Souza Camargo

INSTITUIÇÃO:
Pontifícia Universidade Católica do Paraná

CENTRO / CURSO:
CCBS / Mestrado em Ortodontia

ESPÉCIE DE ANIMAL	SEXO	IDADE / PESO	CATEGORIA	QUANTIDADE
Ratos (*Ratus norvegicus*)	Machos	09 semanas 300-350g	B	75

O colegiado do CEUA em reunião no dia 11/08/2011, avaliou o projeto e emite o seguinte parecer: **APROVADO.**

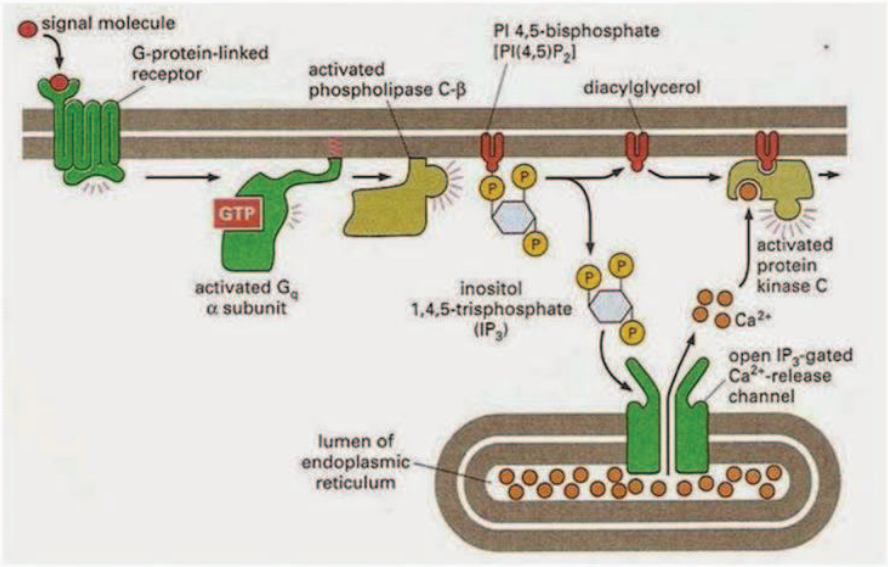
Obs.: Trinta animais são utilizados em conjunto com os projetos 628 e 633 (projeto piloto).

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEUA-PUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas.

Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por este CEUA em qualquer tempo.

Comitê de Ética no Uso de Animais - CEUA
PUCPR

ANEXO B – FIGURA CICLO FOSFATIDILINOSITOL



http://genmol.blogspot.com.br/2014_08_01_archive.html

ANEXO C – NORMAS PARA PUBLICAÇÃO

ARCHIVES OF ORAL BIOLOGY

A Multidisciplinary Journal of Oral & Craniofacial Sciences

AUTHOR INFORMATION PACK

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DESCRIPTION

Archives of Oral Biology operates a web-based submission and review system. Please register at
http://ees.elsevier.com/aob to submit a paper.
Archives of Oral Biology is an international journal which aims to publish papers of the highest scientific
quality in the **oral** and **craniofacial** sciences. The journal is particularly interested in research which
advances knowledge in the mechanisms of **craniofacial development** and **disease**, including:

- **Cell** and **molecular biology**
- **Molecular genetics**
- **Immunology**
- **Pathogenesis**
- **Cellular microbiology**
- **Embryology**
- **Syndromology**
- **Forensic dentistry**

The aim is to be inclusive and multidisciplinary and papers are also welcome in the fields of structure
and function of craniofacial tissues over the whole range of vertebrates including studies concerned
with palaeontology and comparative anatomy. *Archives of Oral Biology* will also publish expert reviews
and articles concerned with advancement in relevant methodologies. The journal will only consider
clinical papers where they make a significant contribution to the understanding of a disease process.

AUDIENCE

Oral biologists, physiologists, anatomists, pathologists.

IMPACT FACTOR

2013: 1.880 © Thomson Reuters Journal Citation Reports 2014
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ABSTRACTING AND INDEXING

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